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AMPLIFICATION OF QUININE CARDIAC EFFECTS BY THE RESISTANCE-REVERSING AGENT PROCHLORPERAZINE IN FALCIPARUM MALARIA

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Abstract. The use of reversing agents to overcome drug resistance is a potential new treatment strategy for both malaria and cancer. Laboratory studies have raised questions about the safety of this therapeutic approach, but data in humans are lacking. We therefore assessed the toxic potential of reversing agent therapy in Thai patients receiving quinine (17 mg/kg given over 4.5 hr) for falciparum malaria by serial measurements of the QT_c interval, an electrocardiographic (ECG) marker of the effect of quinine. Six patients were randomly assigned to receive intravenous quinine alone while another six received one intramuscular injection of 12.5 mg of the reversing agent prochlorperazine (PC; compazine®, stemetil®) 2.5 hr after the quinine infusion had begun. Compared with baseline values at 2.5 hr, there was prolongation of the QT_c interval 30, 60, 90, and 120 min after PC was injected ($P < 0.05$) but no further lengthening with quinine alone ($P > 0.2$). Prochlorperazine alone did not lengthen the QT_c interval in six healthy volunteers. Neither total nor free quinine plasma levels increased after PC was injected, suggesting that ECG changes may have been due to PC-induced intracellular accumulation of quinine. Although only minor quinine ECG effects were amplified by the reversing agent PC in this study, resistance-reversing therapy could potentiate more serious drug effects. The possibility that more serious toxic effects could be produced by this therapeutic approach should be investigated further.

Infection with drug-resistant strains of *Plasmodium falciparum* is a growing public health problem in most malaria- endemic areas. Mechanisms of resistance remain poorly understood, but some resistant parasites can rapidly release antimalarial compounds, thereby reducing drug accumulation.[1] Greater drug efflux is one of several features resistant malaria parasites share with drug-resistant cancer cells, in which the efflux is mediated by a transport protein, P-glycoprotein.[2, 3]

A new, as yet unproven approach to malaria treatment involves reversing resistance by using calcium antagonists and other reversing agents to inhibit the parasite's drug efflux pump.[4] However, the rationale for reversing agent therapy is perhaps compromised by the fact that normal human tissues contain P-glycoprotein,[3, 5] which may pump harmful substances out of cells. If so, a reversing agent could concentrate a co-administered antimalarial drug not only within the parasite but also within normal host tissue. For example, three different reversing agents increased intracellular concentrations of chloroquine in cultured hepatocytes.[6] It has also been observed that when mice were given the anticancer compound vincristine with the reversing agent verapamil, vincristine tissue levels were increased, with more deaths following the combination than in mice given vincristine alone.[7]

We therefore investigated the possible toxicity of reversing agent treatment by measuring delays in myocardial repolarization in patients receiving quinine for falciparum malaria. Such delays, manifested as a prolonged QT_c interval on the electrocardiogram (ECG), occur commonly at therapeutic plasma quinine levels but are not associated with cardiac or other serious adverse effects.[8–10] A prolongation of the QT_c interval is therefore an objective, safe measure of quinine effect. Serial QT_c interval determinations were made in patients receiving quinine alone and compared with values obtained in patients receiving a combination of quinine and prochlorperazine (PC; compazine®, stemetil®). Prochlorperazine is commonly used to control vomiting

in patients with malaria and has reversing agent properties.[11] Our intent was to determine if PC would amplify the electrocardiographic effects of quinine.

PATIENTS AND METHODS

Volunteer patients with uncomplicated falciparum malaria who required parenteral therapy were entered into the study after giving written informed consent. Prospective patients who by history were likely to have taken antimalarials that would still be present in the blood were excluded. Study subjects were randomly assigned to receive one of two regimens by a computer-generated random numbers list. The first regimen (Q) was a constant rate infusion of quinine dihydrochloride given as 7 mg/kg of body weight for 30 min followed by 10 mg/kg for 4 hr.[12] The second regimen (QP) included an identical infusion of intravenous quinine but, in addition, 12.5 mg of PC was given intramuscularly 2.5 hr after the quinine infusion had begun. A control group of healthy Thai male volunteers received a single intramuscular injection of 12.5 mg of PC alone.

Preinfusion ECGs were performed and repeated at 30-min intervals. The QT_c interval was measured without knowledge of the patient's drug regimen from the lead showing greatest T-U distinction at a paper speed of 50 mm/sec. The QT_c intervals were corrected for rate[13] and the mean of five measurements was recorded. Patients were questioned every hour about quinine side effects. Clinical status and vital signs were assessed every 30 min.

For the measurement of quinine concentrations, plasma was obtained from each patient at 0, 2.5, 3.0, 3.5, 4.0, and 4.5 hr after drug administration. The amount of free (non-protein bound) quinine was determined by ultrafiltration using a micropartition system with YMT membranes (Amicon Division, Beverly, MA). Plasma and ultrafiltrate samples were assayed for quinine by high-performance liquid chromatography (HPLC) using fluorescence detection.[14] Plasma specimens for PC analysis detection were taken every half-hour for 2 hr after the drug was given, protected from light, and stored at −70°C. The PC levels were measured by HPLC with electrochemical detection.[15]

Clinical and laboratory findings on admission were compared using the Student's *t*-test for normally distributed values and the Mann-Whitney U test for values not normally distributed. The QT_c intervals at 3.0, 3.5, 4.0, and 4.5 hr were compared using the paired Student's *t*-test to the QT_c interval at 2.5 hr (the time when PC was administered to the QP group). The Spearman's rank test was used to correlate QT_c intervals with plasma PC levels. Two-tailed tests of significance were calculated in all cases.

RESULTS

The initial mean ± SD falciparum parasitemia was 16,413 ± 4,423/mm³ of blood in the Q group and 16,637 ± 4,487 in the QP group ($P > 0.1$). There were no significant differences between treatment groups in the initial QT_c interval, age, body weight, or hematologic indices. Biochemical parameters were also comparable except that mean serum albumin levels were higher in the Q group than in the QP group (4.4 ± 0.1 and 3.9 ± 0.1 g/dl, respectively; $P < 0.05$). Individual albumin levels, however, were all within the normal range.

The QT_c interval increased to a median of 106% of the preinfusion value 2.5 hr after beginning treatment with quinine (range 103–111%). At 2.5 hr, half the malaria patients received intramuscular PC and half did not; the quinine infusion was continued in both groups. Compared with baseline values at 2.5 hr, there was statistically significant further QT_c prolongation 30, 60, 90, and 120 min after injection of PC in the QP group ($P < 0.05$; Figure 1a). The QT_c interval did not change in the Q group ($P > 0.2$; Figure 1a) and became significantly shorter 60, 90, and 120 min after injection of PC in the six healthy volunteers who received this drug alone ($P < 0.05$; Figure 1a).

The prolongations of myocardial repolarization in the QP group could not be explained by a PC-quinine interaction that affected either total or free quinine plasma levels (Figures 1b and c, respectively). The concentration-time curves for total and free quinine in the QP patients were similar to those in the Q group, and quinine concentration curves differed from the QT_c curves (Figure 1). Similarly, plasma levels of PC did not correlate with QT_c intervals during the 2-hr period following injection of PC ($P > 0.2$; Table 1).

No cardiovascular or other side effects occurred in either the 12 malaria patients or the

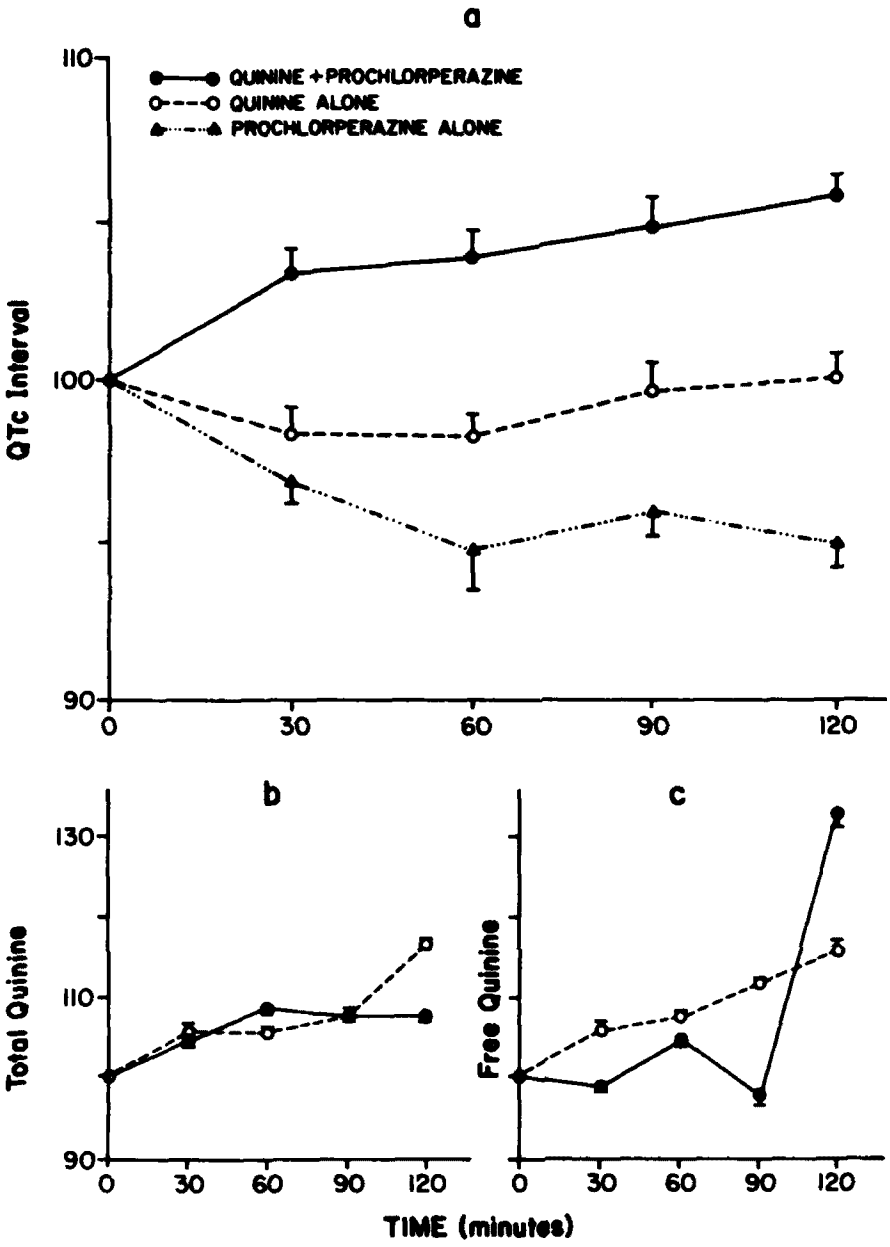

FIGURE 1. a, Changes in the QT_c interval after treatment with either quinine and prochlorperazine (group QP), quinine alone (group Q) or prochlorperazine alone (group P). b, total quinine plasma levels. c, free quinine plasma levels. Bars are the percentage of a baseline value and 95% confidence interval determined after 2.5 hr of quinine infusion (groups QP and Q) or immediately before injection (group P). Note prolongation of the QT_c interval in the group QP only (a) that is not explainable by changes in quinine levels (b and c).

six healthy volunteers. The QT_c intervals lengthened but even our highest recorded value (360 msec) was well below the upper limit of normal values (440 msec). Standard treatment with oral quinine (650 mg per os every 8 hr) and tetracycline (250 mg per os four times a day) was begun as soon as patients were able to tolerate oral medication and produced clinical and parasitologic cures in every case.

DISCUSSION

The rationale for the use of reversing agents in malaria centers on similarities between multidrug resistance in *P. falciparum* infection and that in tumor cells, where resistance is mediated by a transport protein product of the multidrug-resistance (*mdr1*) gene.[2, 3] There is evidence both for and against a role for *mdr*-like genes in re-

TABLE 1

Relationship between plasma prochlorperazine (PC) levels and QT_c intervals in six patients receiving PC and quinine

	Minutes after PC			
	30	60	90	120
Median plasma PC level, ng/ml (range)	4.3 (2.1–5.9)	3.8 (2.3–5.4)	4.2 (2.1–5.4)	4.0 (1.9–4.6)
QT_c interval (% of value before injection of PC	105	108	107	107
Correlation coefficient (r)*	0.77	0.14	0.09	0.43
Significance of correlation (*P*)	0.2	0.5	0.8	0.2

* Spearman's rank correlation between the QT_c interval and the plasma PC level.

sistant *P. falciparum* infection.[16, 17] The possible harmful effects of reversing agent therapy are of concern in both malaria and neoplasia, but are difficult to assess in cancer patients. When compared with individuals being treated for malaria, oncology patients are generally older, are receiving more toxic therapeutic agents, and have multiple medical problems.

The results of our study extend observations from in vitro and animal work, which suggest that reversing agent therapy may be unsafe.[6, 7] Prochlorperazine clearly amplified the delays in myocardial repolarization that commonly accompany quinine treatment. The mechanism(s) by which ECG changes were potentiated by PC cannot be stated with certainty. Quinine levels did not increase after PC was injected (Figure 1b). Serum albumin levels were lower in the QP group, but more biologically active unbound drug was not the explanation for QT_c interval lengthening in these patients. The QP group generally had lower, not higher, levels of free quinine (Figure 1c); α_1 acid glycoprotein, not albumin, is the major protein that binds quinine.[18]

It is unlikely that PC by itself produced the ECG changes. The QT_c interval lengthening is a prominent toxic effect of some phenothiazines, particularly piperidine phenothiazines such as thioridazine.[19] Piperazine phenothiazines, such as PC, are not noted for cardiac effects. The PC levels in plasma were not correlated with QT_c interval duration (Table 1) and PC given alone to healthy Thai controls shortened rather than lengthened myocardial repolarization (Figure 1a).

How did the administration of PC with quinine lead to lengthening of the QT_c interval? One possibility is that PC slows the extrusion of quinine from cardiac tissue, perhaps by inhibition of an export pump such as P-glycoprotein. Prochlorperazine reversed quinine resistance in cultured falciparum parasites (Kyle D, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, unpublished data), presumably by inhibition of the parasite drug pump. Another possibility is that PC potentiates quinine effects on cardiac cell membranes. Quinine, like quinindine, is thought to lengthen the duration of myocardial repolarization by influencing action potentials of cells in the cardiac conduction system,[20] and has been shown to alter cell membrane electrical conductivity.[21]

We measured a benign ECG effect of quinine as a marker of potential toxicity, and observed that quinine co-administered with PC prolonged the QT_c interval. The theoretical concern that inhibition of P-glycoprotein might increase the toxicity of drugs to normal tissues[22] is now supported by clinical evidence. Although methods may be found for overcoming the toxicity of individual reversing agents,[23] concerns about their potentiation of the effects of co-administered compounds should be investigated further. Such concerns are timely since clinical trials of reversing-agent therapy have now begun.[24, 25]

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